Clinical Techniques in Equine Practice

Wound Repair in the Horse: Problems and Proposed Innovative Solutions

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Second intention healing of full-thickness limb wounds in the horse is subject to numerous complications. In particular, an inefficient but protracted inflammatory response may preclude the ensuing proliferative and remodeling phases of the repair process from proceeding normally. Furthermore, the horse seems afflicted with an excessive fibroblastic response leading to the exuberant formation of granulation tissue and subsequent retardation of epithelialization and wound contraction. This article will discuss recent discoveries pertaining to the cellular and molecular mechanisms regulating wound repair in the horse. It will then address innovative solutions that have been implemented in the equine species. Although conservative and time-honored methods of wound care are unlikely to disappear, the development of substances that stimulate repair are imminent and equine practitioners must remain aware of these innovations to better serve their clients and patients.
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Many full thickness wounds in horses are allowed to heal by second intention because massive tissue loss, excessive contamination and skin tension, as well as unacceptable duration since the onset of injury commonly preclude primary closure (Fig. 1). Second intention repair involves the formation of granulation tissue over which epithelium migrates to cover the wound surface, and which eventually contracts to reduce wound size and remodels to improve tissue strength. This type of repair results in the formation of scar tissue which may adversely affect function as well as lead to an unacceptable appearance, particularly for show horses. Although healing often progresses uneventfully in body wounds, repair of full-thickness distal limb wounds in horses is subject to numerous complications such as chronic inflammation and excessive fibroplasia with subsequent retardation of epithelialization and contraction. As such, new therapeutic modalities are actively sought.[1] This article will highlight recent discoveries pertaining to wound repair in the horse, as well as address innovative solutions put forth in the scientific literature.

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Inflammatory Phase

The inflammatory phase is essential to protect against infection as well as to initiate the repair process. Activated tissue macrophages appear to play a key role in the transition between inflammation and repair, through release of multiple cytokines and growth factors that initiate migration and proliferation of the mesenchymal cells involved in angiogenesis, fibroplasia and epithelialization. Paradoxically, prolonged inflammation may contribute to the pathogenesis of a number of diseases characterized by disproportionate scarring, such as hepatic cirrhosis, pulmonary fibrosis, corneal scarring, and dermal keloids. Specifically, chronic wounds differ from acute wounds in that repair occurs with the formation of abundant granulation tissue and often with excessive fibrosis leading to scar contracture which may result in loss of function.[2] It has been shown experimentally that the initial inflammatory response during second-intention healing is weak but protracted in horses compared with ponies,[3] and that horse leukocytes produce less reactive oxygen species essential to bacterial killing[4] (Fig. 2). It appears that the stronger inflammatory response in ponies is more effective under field conditions in preventing wound infection, resulting in a lower incidence of wound dehiscence and bone sequestrum formation.[5] In view of these facts, it is important that the immediate inflammatory response to wounding not be inhibited; as such, the use of NSAIDs should be limited. For example, it has been shown that high doses of NSAIDs used in the early period following creation of linea alba incisions in ponies delay repair.[6]

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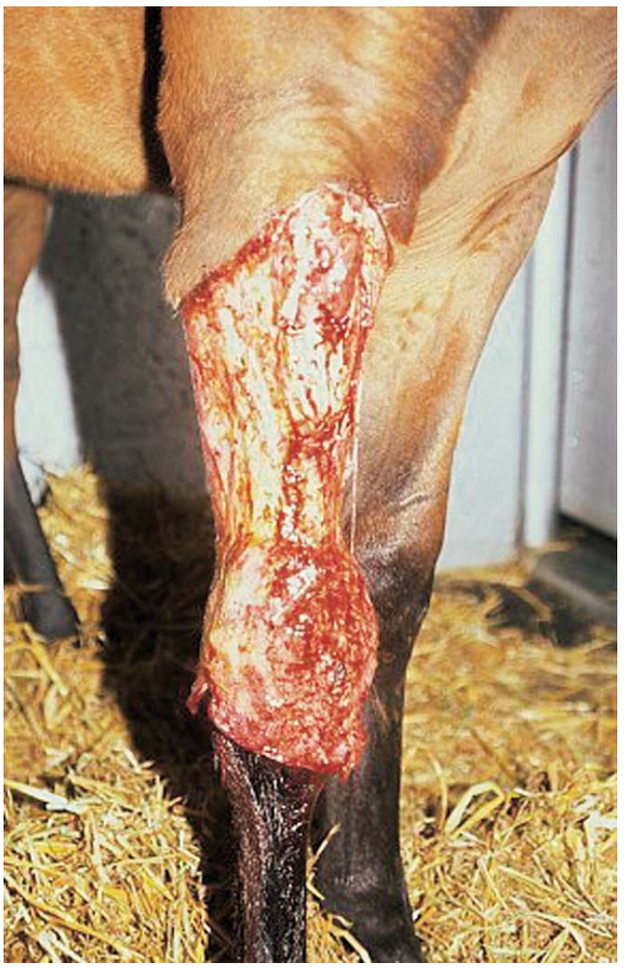

Figure 1 Fresh degloving injury of the forearm. Photo courtesy of FMV, Université de Montréal.

It is not yet possible to stimulate the inflammatory response in a specific way, although a general strategy for improving wound repair may be via any molecule that can recruit or possibly activate macrophages during the acute inflammatory phase. The current trend of applying sugar or honey to open wounds dates back a long time and has recently acquired some scientific merit. Both products are chemoattractant for tissue macrophages and when applied to contaminated or infected wounds may exert antibacterial properties. In particular, honey contains inhibine, an enzyme from bee pharyngeal glands, which breaks down to hydrogen peroxide and glucolactone/gluconic acid; these act as a mild disinfectant and mild antibiotic, respectively. A low pH, high osmolarity as well as the mechanical barrier against bacterial invasion provided by honey's viscous nature ensure a natural antimicrobial effect.[7] Honey also provides antioxidants which protect wound tissues from the damage imparted by free oxygen radicals released from inflammatory cells.[8] Finally, both honey and sugar have been shown to enhance granulation tissue formation and epithelialization, possibly via their stimulatory activity on the tissue macrophage.[9] In particular, it has recently been shown that the stimulatory effect of honey on wound healing may in part be related to the upregulation of inflammatory cytokines (TNF-α, IL-1β, IL-6) within monocytes.[10] A synthetic form of sugar, a D-glucose polysaccharide, Maltodextrin N.F., is commercially available (Intracell®, Macleod Pharmaceutical, Ft. Collins, CO) in hydrophilic powder and gel forms for use as a wound healing stimulant. The medication is reported to be chemoattractant to neutrophils and macrophages, which stimulates the inflammatory phase of healing and thus favors early appearance of granulation tissue as well as rapid epithelial growth.[11] A β(1-4) acetylated mannan, available as a topical hydrogel (Carravet®, Veterinary Products Laboratories, Phoenix, AZ; Carrasorb®, Carrington Laboratories, Irving, TX), likewise enhances the early stages of wound repair by stimulating macrophages to produce TNF-α and IL-1.[12,13] A veterinary study has demonstrated faster healing in dog foot pad wounds treated with acemannan.[14] A hydrophilic freeze dried form of the hydrogel is commercially available; however, it can cause excess formation of granulation tissue, which might limit its usefulness in the horse.[11]

A promising therapy aimed at activating the wound macrophage in the early phase of repair involves the release of iodine from the dressing. Iodosorb® dressing comprises particles manufactured from cross-linked polymerized dextran containing iodine. (Iodosorb®, Smith & Nephew, Hull, UK) As the dressing becomes hydrated in the moist wound environment, elemental iodine is released to exert an antibacterial effect and to interact with macrophages to modulate production of TNF-α and IL-6 and thus indirectly influence the repair process.[15] Although the use of Iodosorb® dressings has not been reported in horses, Wilson studied the effects of activated macrophage supernatant on distal limb wounds of ponies, citing a similar rationale.[16] It was postulated that the supernatant contained a wide variety of cytokines important in the inflammatory process and secreted by the activated inflammatory cells. Unfortunately, it was found that the macrophage supernatant exerted no significant effects on healing in vivo. Another equine study aimed at enhancing the acute inflammatory response during repair of deep wounds evaluated the efficacy of Solcoseryl®, a protein-free, standardized dialysate/ultrafiltrate derived from calf blood. (Solcoseryl®, Solco Basle Ltd, Birsfelden, Switzerland)[17] In the first month of repair, Solcoseryl® provoked a greater inflammatory response, with faster formation and contraction of granulation tissue. Subsequently, it inhibited repair by causing protracted inflammation and delaying epithelialization. The authors thus recommend its use in the treatment of deep wounds during the initial inflammatory phase of second intention repair; treatment should be ceased when epithelialization predominates. A controlled field study was recently performed to determine the efficacy of Vulketan gel® in preventing exuberant granulation tissue formation in equine lower limb wounds (Janssen Animal Health, Beerse, Belgium).[18] Ketanserin, the active ingredient in the gel, is thought to antagonize the serotonin-induced suppression of wound macrophages and thus allows a strong and effective inflammatory response to occur within wounds. This should translate into a superior control of infection and a better orchestration of the later phases of repair when the cytokines and growth factors released by the activated macrophages play an important role. Vulketan was two to five times more likely to result in successful closure by reducing infection and proud flesh formation, than an antiseptic or a desloughing agent.

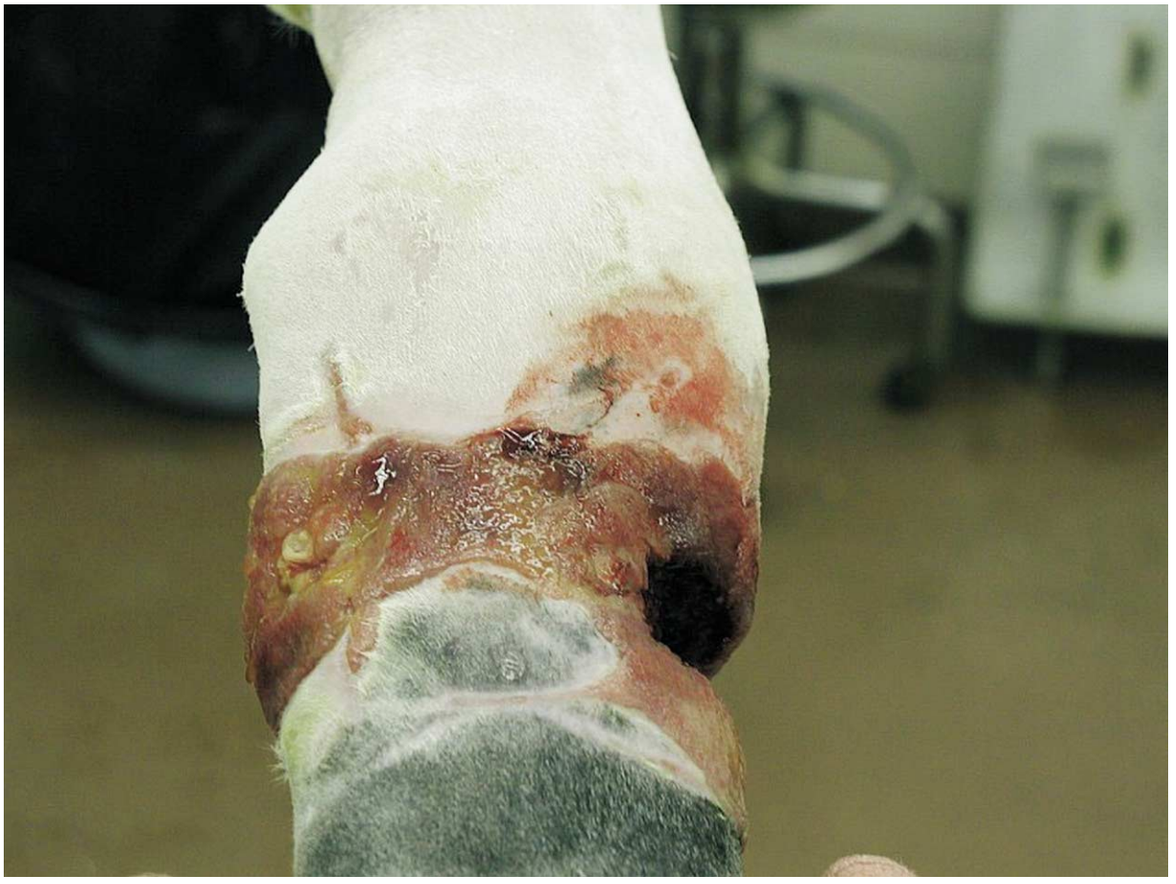

Figure 2 Circumferential wound just proximal to the fetlock, caused by metal sheeting, and involving both flexor tendons as well as their synovial sheath. Chronic inflammation related to infection and persistence of frayed tendon ends. Photo courtesy of FMV, Université de Montréal.

Debridement is an important step in the initial treatment of a deep wound, particularly when necrosis, exposed cortical bone or frayed tendons are present (Fig. 3). This will assist with the demarcation of nonviable tissue and reduce the total length of the inflammatory phase. Although debridement can be achieved through surgical, bandage, enzymatic, laser or biosurgical means, equine practitioners have traditionally relied on the first two methods. Dart and colleagues examined the efficacy of a 25% propylene glycol hydrogel (Solugel®, Johnson and Johnson Medical, North Ryde, Australia) on second intention wound healing in horses.[19] By enhancing the moisture content of necrotic tissue and increasing collagenase production within the wound, hydrogels facilitate autolytic debridement. Unfortunately, Solugel® did not appear to have any beneficial effect on healing of small full-thickness skin wounds on the distal limb of horses.

Proliferative Phase

The proliferative phase of repair involves angiogenesis, fibroplasia and epithelialization. For some time it has been known that the horse activates wound collagen formation to a greater extent and earlier during repair than do other species.[20] This predisposes it to the formation of exuberant granulation tissue (proud flesh), with subsequent retardation of contraction and epithelial migration. Furthermore, limb wounds take significantly longer to heal than do body wounds, as a result of a prolonged inflammatory phase as well as deficient wound contraction.[21] Specifically, greater wound retraction, deficient epithelialization, and excessive formation of unhealthy granulation tissue commonly afflict limb wounds.[22] In most species, fibroplasia and epithelialization are favored by a moist wound environment such as that provided under certain bandages, while in the horse occlusive dressings result in significantly prolonged healing times and production of excess wound exudate and granulation tissue.[23,24] A notable exception is achieved with the use of amnion, a biological, species-specific, nonadherent wound dressing material, both in second intention healing of wounds located at the distal aspect of the limb,[25] where it enhances epithelialization and accelerates closure, and in pinch-grafted wounds.[26] For more information on amnion refer to the article "Update on Wound Dressings" in this issue.

Aside from the aforementioned Vulketan gel®, various topical agents may be used during the proliferative phase of repair, in an attempt to encourage healthy fibroplasia. Glycyl-L-histidyl-L-lysine tripeptide-copper complex (TPCC) is a topical wound healing stimulant with chemotactic properties for inflammatory cells, which provide cytokines and growth factors to stimulate the proliferative phase of repair (Iamin-vet®, Procyte, Redmond, WA). It has been shown to enhance production of several extracellular matrix components, including collagen type I, proteoglycans (chondroitin sulfate and dermatan sulfate) and glycosaminoglycans (bigly-

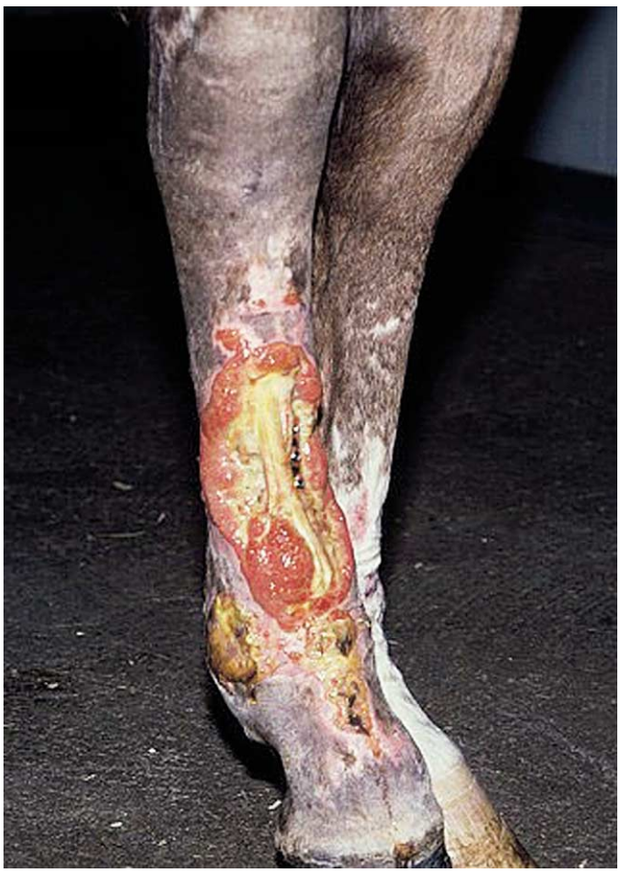

Figure 3 Wound at dorsal aspect of metacarpus, with exposed bone. Debridement is warranted. Photo courtesy of FMV, Université de Montréal.

can and decorin) during wound repair.[27] Furthermore, it appears that TPCC not only activates the proliferative phase of repair, but may also contribute to the remodeling phase via its ability to modulate expression of certain matrix metalloproteinases and their inhibitors.[28]

Excessive fibroplasia has been the topic of many equine studies, some focusing on its pathophysiology and others aiming to prevent its development (Fig. 4). In view of the fact that cytokines and growth factors are critical mediators of the repair process, investigators have recently attempted to document their involvement in wound repair in the horse. Thus, it has been shown that expression of pro-fibrotic TGF-β1 persists throughout the proliferative phase of healing in wounds of the limb whereas it quickly returns to baseline values in thoracic wounds after the initial inflammatory phase.[29-31] The wound macrophage and fibroblast have been incriminated for this augmented TGF-β1 synthesis,[32] while strong expression of TGF-β receptors in limb wounds, particularly those developing proud flesh, suggests that the signaling machinery for stimulation of matrix proteins is in place to contribute to scarring.[33] Finally, preliminary studies suggest that natural[34] or experimental[35] wounds healing with exuberant granulation tissue are influenced by higher concentrations of pro-fibrotic TGF-β1 and lower concentrations of antifibrotic TGF-β3 compared with those healing normally.

Since problematic wound repair, including chronicity and fibrosis, may result from excessive inflammation and an abnormal cytokine profile, investigators have attempted to alter this balance in hopes of ameliorating the quality of repair. Topical application of TGF-β has been shown to improve repair in a variety of animal models of chronic, impaired wound healing. A study by Steel tested two concentrations of a recombinant form of the pro-fibrotic isoform, TGF-β1, on full-thickness wounds located at the distal aspect of the limb of horses.[36] There were no beneficial effects on total amount of granulation tissue, epithelialization area, or on histologic or subjective clinical assessments of wound biopsies. Conversely, Ohnemus has achieved promising results by topically applying the antifibrotic isoform, TGF-β3, to wounds created at the distal aspect of the limb in horses.[37] Although the small number of subjects obviated statistical significance of that study, preliminary data suggest that the growth factor encourages formation of healthier granulation tissue that does not become exuberant, despite the use of bandages.

Topical application of corticosteroids to halt excessive fibroplasia is somewhat controversial. Continued application is not recommended since it may also exert negative effects on wound contraction, epithelialization and angiogenesis.[38] However, it is interesting to note that some corticosteroids selectively decrease the release of pro-fibrotic TGF-β1 and β2 from monocytes and macrophages.[39] If corticosteroids are used, it is suggested that one or two applications at the first signs of excessive fibroplasia is all the is needed.

Wounds that exhibit delays in epithelialization often develop hypertrophic scarring in humans. Thus, it is critical that epithelialization proceed in a speedy fashion. As its name would imply, epidermal growth factor is known to enhance epithelialization via positive effects on epithelial cell migration, proliferation and differentiation. This growth factor has recently been applied topically to experimentally induced corneal wounds of horses in hopes of accelerating epithelialization. Unfortunately, it was found that beneficial effects were outweighed by the intensity of the associated inflammatory response, at least in the eye.[40]

Wound repair is the result of complex interactions among blood constituents, soluble mediators such as cytokines and growth factors, cells and extracellular matrix components. It is thus not surprising that the overall clinical experience with cytokines and growth factors used exogenously to improve repair has been discouraging.[41] Likewise, it is unlikely that application of a single cytokine would mimic natural processes and enhance repair unless impairment was due to the relative lack of that specific agent. Thus, it is possible that combinations of various cytokines/growth factors would act synergistically and subsequently promote more effective healing. Following this logic, cell extracts and supernatants from epithelial cells were topically applied to chronic granulomas on horse limbs. Although the active ingredient remains unidentified, the study provided promising results: epithelialization was accelerated and fibroplasia was held in check.[42] More recently, an all natural equine-specific wound healant (Lacerum®, BeluMedX, Little Rock, AK), advertised as containing activated platelets and their released growth factors, has been shown to induce repair in injuries previously deemed untreatable.[43] Along those same lines, elk velvet antler extract, comprising various growth factors and known to stimulate fibroblast growth in vitro[44] has been shown to ac-

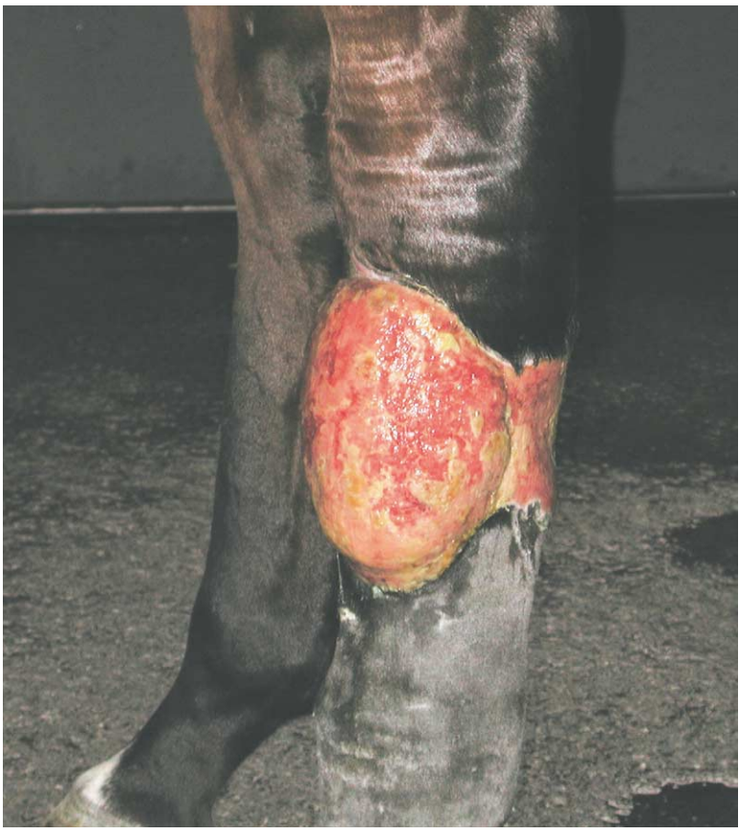

Figure 4 Exuberant granulation tissue, elevated above the skin edges and projecting over the advancing border of epithelium. Photo courtesy of FMV, Université de Montréal.

celerate wound repair in diabetic rats by topical application.[45] According to the premise that slow growth of fibroblasts from equine limbs could contribute to the poor healing characteristics of wounds in this area,[46] this extract may be an economical adjunct to the treatment of full-thickness limb wounds in horses.

At the Université de Montréal we are currently investigating the efficacy of a silicone gel dressing (CicaCare®, Smith & Nephew, Hull, UK) in preventing the development of proud flesh in wounds located at the distal aspect of the limb in horses (Fig. 5). This treatment is successful in reversing hypertrophic scarring in human burn patients, apparently by exerting pressure on the microvasculature of the scar[47] and altering levels of various growth factors, notably pro-fibrotic TGF-β.[48] The anoxic fibroblasts are then thought to undergo apoptosis rather than proliferating and secreting extracellular matrix components. We observed that the silicone gel dressing greatly surpassed a conventional nonadherent absorbent dressing in preventing the formation of exuberant granulation tissue in experimental wounds. Contraction and epithelialization progressed faster in the first two weeks of repair, possibly as a result of the healthier granulation tissue. Furthermore, tissue quality exceeded that of wounds treated conventionally, which may translate into superior tissue strength.[49] Although no single dressing is suitable for all stages of all wounds, the information gleaned from this study may assist in designing an appropriate wound management strategy to prevent fibrosis and unsightly scarring of wounds at the distal aspect of limbs in horses.

Various biomaterials are commercially available to support ingrowth of mesenchymal cells during the proliferative phase of repair. For instance, collagen membranes and sponges have been associated with improved rates of healing and wound appearance in experimental animals. The collagen is thought to function as a substrate for hemostasis and, in addition to serving as a template for cellular attachment, migration and proliferation, may promote wound maturation by providing a scaffold for more rapid transition to mature collagen production and alignment. A porous bovine collagen membrane has been tested in full-thickness limb wounds in horses.[50] Although it appeared to result in a greater degree of inflammation than the control dressing, which may have augmented cytokine/growth factor release, it did not significantly alter the total wound, epithelialized or contraction areas. A commercially available natural and biocompatible collagen matrix, derived from porcine small intestinal submucosa (Vet BioSISt®, Cook Veterinary Products Inc, Spencer, IN) and containing a plethora of proteins and growth factors, is designed as a scaffold for tissue ingrowth and is touted to reduce scarring. Regrettably, a recent study determined that it offers no apparent advantage over a nonbiological, nonadherent synthetic dressing for treatment of small, granulating wounds of the distal limb of horses.[51] Another commercially available acellular bioscaffold derived from porcine urinary bladder is presently being evaluated for use in small and large animals. For more information refer to "Update on Wound Dressings" in this issue. Skin substitutes are increasingly being used in the treatment of difficult to

Figure 5 Silicone gel dressing (CicaCare® by Smith-Nephew).

heal wounds in humans. A handful of recent FDA-approved biomaterials are bioengineered human skin grafts derived from cultured dermal cells (Dermagraft® and TransCyte®, both from Smith & Nephew, Hull, UK). Similar to a full-thickness skin graft, these living bilayer grafts have cellular and growth factor components along with the full complement of matrix proteins essential to dermal repair. Indeed, it has been demonstrated that they are a truly dynamic living tissue, capable of responding to physical injury in a staged and specific pattern of cell migration, epithelialization, and cytokine expression.[52]

Remodeling Phase

The final phase of repair involves reduction of wound size by contraction, and reorganization of the extracellular matrix components synthesized during the preceding phase. Wound contraction accelerates closure and also enhances the cosmetic appearance and strength of the scar since proportionately less wound area must be covered by fragile neoepithelium. Wound contraction is significantly more pronounced in ponies than in horses, and is more prominent in body than limb wounds.[22] Wilmink recently conducted an in vitro study to determine whether variation in the contractile ability of a wound is due to differences in the inherent contraction capacity of the fibroblasts or to differences in tissue environmental factors. She found that myofibroblasts from body and limb origin did not differ in their innate contraction capacity. Rather, it was felt that tissue environmental factors emanating from the inflammatory response to injury, such as cytokine/growth factor profiles, were instrumental in causing this difference.[53] While the prospect of manipulating wound contraction in the horse has long been enticing, no clinical studies have yet been conducted in this area.

Although deposition of type I collagen bundles peaks 1-2 weeks following wounding, collagen continues to accumulate slowly for another week thereafter. At this time, synthesis is balanced by degradation and the remaining collagen is remodeled indefinitely. Since the ultimate tensile strength of a wound is related to its collagen content, therapies that favor its synthesis and deposition are continuously sought. Growth hormone is postulated to stimulate collagen synthesis by fibroblasts and accelerate its maturation resulting in enhanced wound strength, effects that are probably mediated through various growth factors. A recent study investigating the effect of intramuscular injections of equine recombinant growth hormone (rEGH) on maturation of limb wounds in horses found that the wounds retracted further and contracted at a faster rate only after rEGH treatment ended.[54] A recent study suggests that an imbalance between collagen synthesis and degradation during the remodeling phase of repair likely correlates with the development of exuberant granulation tissue in horse limb wounds.[31] Factors that inhibit collagen synthesis or stimulate matrix metalloproteinases may provide treatment options for these patients.

In conclusion, although the premises guiding the previously cited clinical studies are sound, results have been somewhat equivocal. A more precise understanding of the mechanisms of wound repair and scarring in the horse is needed before successful therapeutic approaches can be implemented. In the interim, conservative and time-honored methods of wound care should be attempted first. Further refinement and development of substances that stimulate repair are imminent, and equine practitioners must remain aware of these innovations to better serve their clients and patients.

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